Copper(II) and Nickel(II) Complexes of Sulfhydryl and Imidazole Containing Peptides: Characterization and a Model for "Blue" Copper Sites

Yukio Sugiura* and Yoshinobu Hirayama

Contribution from the Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan. *Received August 10, 1976*

Abstract: The Cu(II) and Ni(II) complexes of new sulfhydryl and imidazole containing peptides such as *N*-mercaptoacetyl-L-histidine (MAH) and 2-mercaptopropionyl-L-cysteine, have been characterized by visible, CD, ^{1}H NMR, and ESR spectra. Potentiometric measurements showed that the formation constant (log K_1K_c) of their Cu(II) and Ni(II) complexes increases in the order $NN_PN_{Im} < SN_PO < SN_PN_{Im} < SN_PS$ donor sets. The 1:1 MAH-Cu(II) complex, which shows an intense absorption near 600 nm (ϵ 830) and a small copper hyperfine coupling constant ($A_\parallel$ = 93 G), has similar unique spectral characteristics to "blue" copper proteins. The model complex strongly suggests that "blue" copper sites involve cysteine sulfhydryl and histidine imidazole coordinations and that the intense 600-nm band is attributed to $\sigma S \rightarrow d(Cu)$ charge transfer transition.

"Blue" copper proteins, which occur widely in nature as an electron carrier, have attracted particular attention because of their unique properties. The distinctive features of "blue" copper centers are unusually high extinction coefficients near 600 nm (ϵ 1000–5000), anomalously small copper hyperfine coupling constants ($A_\parallel$ = 30–100 G), and markedly positive copper redox potentials (E_0' = 0.2–0.8 V).[1] However, the ligand environment of "blue" copper proteins has never been established. Recent resonance Raman[2] and x-ray photoelectron[3] studies suggested the presence of Cu(II)–S(Cys) coordination for "blue" copper sites and S → Cu(II) charge transfer transition for ~600-nm electronic absorption bands. Sulfur–copper bonding in "blue" copper chromophore has also been indicated from the experiments on model Cu(II) complexes of sulfhydryl containing peptides[4] and polythiaethers.[5] On the other hand, ^{1}H NMR spectra at 250 MHz of plastocyanins revealed that the imidazole groups of histidine residues are liganded directly to the copper.[6] We report here the characterization of sulfhydryl containing peptide–Cu(II) and –Ni(II) complexes which involve sulfhydryl, deprotonated peptide nitrogen, and imidazole groups as the coordination sites. Of special interest is the *N*-mercaptoacetyl-L-histidine–Cu(II) complex as a model for "blue" copper sites.

Experimental Section

2,3-Dimercaptopropionylglycine, 2-mercaptopropionyl-L-cysteine, *N*-mercaptoacetyl-L-histidine, and 3-mercaptopropionyl-L-histidine were synthesized by the Schotten–Bauman reaction between 2,3-dibromopropionic acid chloride (or 2-bromopropionic acid chloride, bromoacetic acid chloride, 3-bromopropionic acid chloride) and glycine (or L-cysteine, L-histidine) followed by a condensation with thiobenzoic acid and then hydrolysis in an ammonia solution.[7] The peptide ligands were recrystallized with ethyl acetate and determined by elemental analysis, iodometric titration, and ^{1}H NMR measurement. A freshly prepared solution of the ligands was used in each experiment. The metal solution (0.01 M) was prepared by dissolving cupric nitrate or nickel nitrate in water and standardized with 0.01 M EDTA solution. A carbonate-free potassium hydroxide solution (0.1 M) was prepared by the method of Armstrong[8] and standardized with potassium hydrogen phthalate. All other reagents used were of commercial reagent grade, and deionized water was used throughout.

Visible absorption and CD spectra were measured in an aqueous solution at 20 °C with a Shimadzu recording spectrophotometer, Model Double-40R, and a Jasco J-20 spectropolarimeter, respectively. ^{1}H NMR spectra were recorded at 100 MHz on a Varian HA-100 NMR spectrometer. Sample concentration was 0.2 M in D_2O (pD 9.6) and chemical shifts were measured from internal TSP. X-band ESR spectra were obtained at 77 and 293 K with a Jeol ME-3X spectrometer equipped with a Gauss meter and frequency counter. Potentiometric pH titration was carried out as follows: exactly equimolar amounts (0.004 M) of the ligand and Cu(II) (or Ni(II)) ion were mixed in 18 ml of water and to this was added 2 ml of 1 M KNO_3 to make the total volume of 20 ml. The mixture was titrated with 0.1 M KOH solution at 20 °C under a nitrogen atmosphere, and the pH measurements were carried out with a Radiometer titrator, Type TTT-1C. The formation and dissociation constants of the peptide–metal complexes were calculated according to the previously reported method.[4,9]

Results

Visible Absorption and CD Spectra. Sulfhydryl containing peptides react with Ni(II) and Cu(II) ions to form unique 1:1 complexes in which the deprotonated peptide nitrogen group participates in coordination with the metal. Tables I and II summarize visible absorption and CD spectral data for Ni(II) and Cu(II) complexes of the sulfhydryl containing peptides and the related ligands. The λ_{max} of the 1:1 Ni(II) complexes shifts to a longer wavelength in the order 2-MPC > MAH > 2-MPG, consistent with the order of the formation constants. DMPG forms the 2:1 complex in addition to the 1:1 complex. The 3-MPH–Ni(II) complex gives λ_{max} value similar to that of the 3-MPG–Ni(II) complex. The result suggests that the terminal carboxylate group rather than the imidazole group of 3-MPH participates in Ni(II) coordination. In the 1:1 Cu(II) complexes, on the other hand, the λ_{max} near 600 nm shifts to a shorter wavelength in the order 2-MPC < MAH < 2-MPG. The DMPG–Cu(II) complex has a similar visible spectrum to that of the 2-MPG–Cu(II) complex. A striking feature is the high extinction coefficient of the MAH–Cu(II) complex at ~600-nm band. The diamagnetic Ni(II) complex of 2-MPC, which is composed of L-cysteine residue, typically exhibits a negative CD extremum at 482 nm, whereas the positive CD extremum appears at 463 nm in the MAH–Ni(II) complex in which the histidyl residue is involved in the chelate ring. The MAH–Cu(II) complex shows a unique CD spectrum with the sign (− + −) above 440 nm. In general, the Ni(II) and Cu(II) complexes of L-cysteine and L-histidine containing peptides give a CD curve which has a greater magnitude.

Potentiometric Titration. The titration curve of MAH consists of three pH buffer zones, which give the values of pK_1(COOH, 3.43), pK_2(imidazole, 7.14), and pK_3(SH, 8.70). The acid dissociation constants of 2-MPC are pK_1(COOH, 3.66), pK_2(>CHSH, 7.66), and pK_3(–CH_2SH, 10.69). The titration curves of 1:1 MAH (or 2-MPC)–Ni(II) and –Cu(II) systems gave clearly an inflection at a = 4.0 (a = moles of base per mole of ligand). The data strongly indicate that a proton

Table I. Visible Spectral Data for Ni(II) and Cu(II) Complexes of Sulfhydryl Containing Peptides

Ligand	Absorption max. nm (ε)			
	Ni(II) complex		Cu(II) complex	
2,3-Dimercaptopropionylglycine	440 (1300)	575 (440)	400 (sh)	605 (280)
	455 (150)	605 (100)[a]		
2-Mercaptopropionyl-L-cysteine	410 (1200)	567 (450)	405 (700)	580 (280)
N-Mercaptoacetyl-L-histidine	382 (770)	543 (240)	438 (970)	598 (830)
3-Mercaptopropionyl-L-histidine	405 (850)	515 (250)		
2-Mercaptopropionylglycine	375 (2750)	475 (410)	400 (sh)	605 (260)
3-Mercaptopropionylglycine	400 (1020)	510 (290)	400 (sh)	630 (100)
2,3-Dimercapto-1-propanol[10]	475 (120)	610 (80)[a]		
	452 (3100)	600 (670)[b]		

The composition of the metal complexes is L/M = 1:1, except for [a] L/M = 2:1 and [b] L/M = 3:2, respectively.

Table II. Circular Dichroism Data for Ni(II) and Cu(II) Complexes of Sulfhydryl Containing Peptides

Complex	Circular dichroism λ_{max}, nm ($\Delta\epsilon$)
2-Mercaptopropionyl-L-cysteine-Ni(II)	390 (0.39), 440 (−2.16), 482 (−4.30), 565 (2.88)
N-Mercaptoacetyl-L-histidine-Ni(II)	385 (−1.12), 463 (4.21), 560 (−2.27)
2-Mercaptopropionyl-L-cysteine-Cu(II)	395 (−0.82), 575 (0.59)
N-Mercaptoacetyl-L-histidine-Cu(II)	365 (−3.49), 405 (−1.67), 445 (−1.97), 545 (1.58), 630 (−0.64)
Glycyl-L-histidine-Ni(II)[11]	430 (2.08), 488 (−1.92)
Glycyl-L-histidine-Cu(II)[12]	485 (−0.16), 565 (0.12)
L-Cysteine-Ni(II) (2:1)[11]	390 (−0.11), 455 (−0.62), 535 (0.45)
L-Cysteine methyl ester-Ni(II) (2:1)[11]	420 (−0.03), 500 (0.19), 580 (0.22)
N-Acetyl-L-cysteine-Ni(II) (2:1)[11]	400 (0.35), 460 (−0.07), 525 (−0.20), 610 (−0.04)

Table III. Acid Dissociation and Formation Constants for Ni(II) and Cu(II) Complexes of Sulfhydryl Containing Peptides and the Related Ligands

Ligand	Acid dissociation and formation constants								
				Ni(II) complex			Cu(II) complex		
	pK_1	pK_2	pK_3	$\log K_1$	pK_c	$\log K_1K_c$	$\log K_1$	pK_c	$\log K_1K_c$
2,3-Dimercaptopropionylglycine	3.66	7.66	10.69	10.91	5.89	5.02	7.0	5.3	1.7
				$\log K_1K_2 = 21.53$[a]					
2-Mercaptopropionyl-L-cysteine	3.29	8.48	10.22	11.38	6.16	5.22	14.3	5.7	8.6
N-Mercaptoacetyl-L-histidine	3.43	7.14	8.70	6.57	6.53	0.04	10.6	5.5	5.1
3-Mercaptopropionyl-L-histidine	3.48	7.25	9.86	4.67	7.61	−2.94			
2-Mercaptopropionylglycine	3.60	8.74		5.44	6.88	−1.44	7.6	6.2	1.4
3-Mercaptopropionylglycine	3.71	9.60		4.49	7.63	−3.14	6.9	7.0	−0.1
2,3-Dimercapto-1-propanol[10]	8.69	10.72		$\log K_1K_2 = 22.78$[a]					
Glycyl-L-histidine[13]	2.75	6.85	8.33	3.82	6.10	−2.28	5.08	4.00	1.08
β-Alanyl-L-histidine[14]	<3.0	6.76	9.36	5.42	9.14	−3.72	5.01	4.65	0.36

The composition of the metal complexes is L/M = 1:1, except for [a] L/Ni = 2:1.

from the peptide linkage dissociates in the course of the complex formation and the ligand behaves as a terdentate (or tetradentate) ligand. The acid dissociation and formation constants for the Ni(II) and Cu(II) complexes of DMPG, 2-MPC, MAH, and 3-MPH are listed in Table III together with those of the related complexes. The equilibrium constants K_1 and K_c are defined as $K_1 = [ML]/[M^{2+}][L^{2-}]$ and $K_c = [MA^-]/[H^+][ML]$. Accordingly, the relative stability among the negative complexes (MA^-) at a constant pH is discussed from the following relationship

$$[MA^-]/[M^{2+}][L^{2-}] = K_1K_c/[H^+]$$

where L^{2-}, ML, and MA^- denote respectively the free ligand, the neutral complex, and the negative complex formed upon the dissociation of the peptide NH of ML. As shown in Table III, the log K_1K_c values of the Ni(II) and Cu(II) complexes increase in the order $NN_PN_{Im} < SN_PO < SN_PN_{Im} < SN_PS$ donor sets. The formation constant of 2:1 DMPG-Ni(II) complex is parallel to that of 2:1 2,3-dimercapto-1-propanol-Ni(II) complex, suggesting the formation of a square-planar complex with an S_4 donor set. The DMPG-Cu(II) complex has a log K_1K_c value similar to that of the 2-MPG-Cu(II) complex, which coordinates with Cu(II) through the sulfhydryl, deprotonated peptide nitrogen, and terminal carboxylate groups.[4]

^{1}H NMR Spectra. Figure 1 shows typical ^{1}H NMR spectra of 2-MPC and its 1:1 Ni(II) complex at pD 9.6. The methylene protons of 2-MPC were observed as a single peak (doublet) at 2.93 ppm. On the other hand, the methylene protons of the 1:1 2-MPC-Ni(II) complex were observed as two signals at 2.68 and 2.28 ppm. Thus one has evidence that two protons of the methylene group are in a different environment induced by the formation of a complex with a rigid fused-chelate ring formation. Similar proton splitting of the methylene protons was

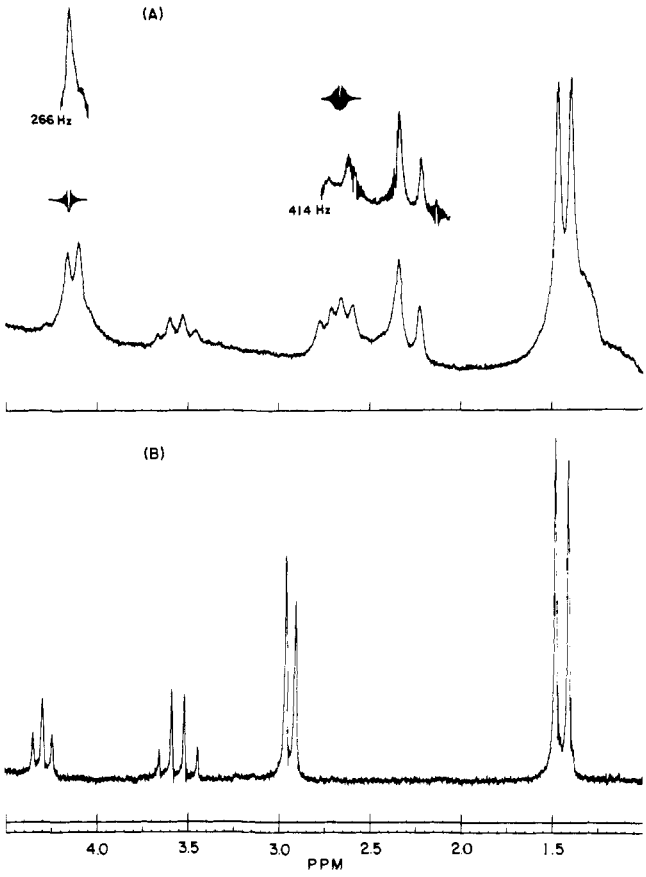


Figure 1. 100 MHz ^{1}H NMR spectra of 1:1 2-mercaptopropionyl-L-cysteine–Ni(II) complex (A) and 2-mercaptopropionyl-L-cysteine only (B) at pD 9.6. In the case of (A), the decoupling results at ω = 266 and 414 Hz are also presented. Chemical shifts are measured from internal TSP.

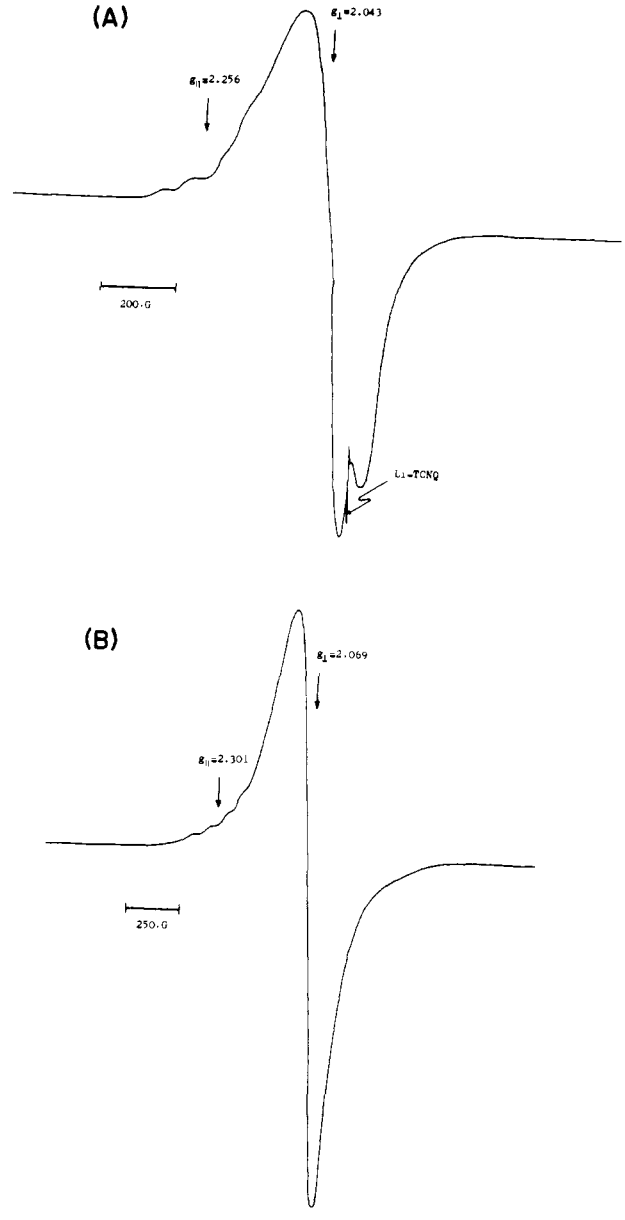


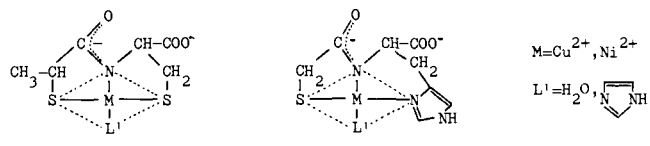
Figure 2. ESR spectra for Cu(II) complexes of 2-mercaptopropionyl-L-cysteine (A) and *N*-mercaptoacetyl-L-histidine (B). Conditions of ESR spectroscopy: microwave power, 8 mW; frequency, 9.198 GHz; modulation amplitude, 6.3 G; time constant, 1.0 s; temperature, 77 K. The spectra were measured by mixing the ligand (10.0 mM) and $CuCl_2$(10.0 mM) in borate buffer of pH 9.2.

obtained in the 1:1 Ni(II) complexes of MAH, 2-MPG, and 2-mercaptopropionylglycinamide. Of the two methylene protons of these Ni(II) complexes, the proton at lower field is considered to be in a pseudo-equatorial position nearly trans to the Ni(II), while the proton at higher field would possess a more axial character.[15] The results of proton chemical shifts of the sulfhydryl containing peptides and their Ni(II) complexes are summarized in Table IV. The α_1 and α_2 protons of the 2:1 DMPG–Ni(II) complex appear at 2.57 (doublet) and 3.37 (triplet) ppm, and the large high-field shift is based on the formation of square-planar S_4–Ni(II) complex with π-bonding character. The proton chemical shift of Im_2 in the MAH–Ni(II) complex indicates the coordination of the histidine imidazole group. In the 1:1:1 2-MPC–Ni(II)–imidazole system, the proton chemical shifts of Im_4 and Im_2 reveal clearly the formation of the imidazole adduct Ni(II) complex.

ESR Spectra. The ESR spectra of Cu(II) complexes of 2-MPC and MAH observed in frozen solution at 77 K are shown in Figure 2. The 2-MPC- and MAH-Cu(II) complexes revealed ESR spectra which represent 92 ± 6 and 95 ± 4% of total Cu by comparison to a Cu(II) standard, respectively.[16] Both ESR spectra exhibit a typical copper hyperfine pattern with approximately axial symmetry, i.e., $g_x \simeq g_y$. The ESR parameters are $A_\parallel$ = 76 G, $g_\parallel$ = 2.256, and $g_\perp$ = 2.043 for the 2-MPC–Cu(II) complex, and $A_\parallel$ = 93 G, $g_\parallel$ = 2.301, and $g_\perp$ = 2.069 for the MAH–Cu(II) complex. The ESR absorptions at g = 4 (ΔM_s = 2) due to triplet dimer have never been detected, and the ligand superhyperfine stucture has never been observed regardless of the experimental conditions. The values for $g_\parallel$, $g_\perp$, and $A_\parallel$ of these complexes are typical for the Cu(II) complexes. As to the copper hyperfine coupling constant in the parallel region, these complexes have a considerably small $A_\parallel$ value, which suggests a decrease in the unpaired electron density on the Cu(II) atom.

Discussion

From the present results, the following coordination mode is reasonably proposed for the 1:1 Cu(II) and Ni(II) complexes of 2-MPC and MAH. Of special interest is the MAH–Cu(II)



complex[17] as a model for "blue" copper proteins. As shown in Figure 3, the MAH–Cu(II) complex exhibits an intense absorption band in the 600-nm region as well as an additional band near 440 nm. The ratio of $\Delta\epsilon$ to ϵ is of the order 10^{-3} for

Table IV. Proton Chemical Shifts of Sulfhydryl Containing Peptides and Their Ni(II) Complexes

Compd	Proton chemical shift, ppm					
	α_1	α_2	α_3	β	Im_4	Im_2
$CH_2CHCONHCH_2COOH$ (SH, SH)	2.94	3.65	3.81			
Ni(II) complex (2:1)	2.57	3.37	3.82			
Ni(II) complex (1:1)	2.62	3.55	3.80 (J = 5.0 Hz)			
$CH_3CHCONHCHCH_2SH$ (SH, COOH)	1.44	3.53	4.29	2.93		
Ni(II) complex (1:1)	1.42	3.59	4.13	2.68 (J = 5.5 Hz) 2.28 (J = 12.0 Hz)		
Imidazole					7.14	7.79
Imidazole adduct Ni(II) complex	1.45	3.57	4.18	2.72 (J = 5.5 Hz) 2.32 (J = 12.0 Hz)	6.97 6.86	7.64
$CH_2CONHCHCH_2$ (imidazole) (SH, COOH)	3.20	4.42		3.08	6.99	7.78
Ni(II) complex (1:1)	3.33	4.46		3.07 (J = 3.5 Hz) 2.81 (J = 8.0 Hz)	6.96	7.69
$CH_2CH_2CONHCHCH_2$ (imidazole) (SH, COOH)	2.70	2.62	4.49	3.05	6.97	7.74
Ni(II) complex (1:1)	not resolved for broadening					
$CH_3CHCONHCH_2COOH$ (SH)	1.46	3.59	3.77			
Ni(II) complex (1:1)	1.34	3.62	3.79 3.36			
$CH_3CHCONHCH_2CONH_2$ (SH)	1.43	3.57	3.91			
Ni(II) complex (1:1)	1.42	3.77	3.96 3.31			

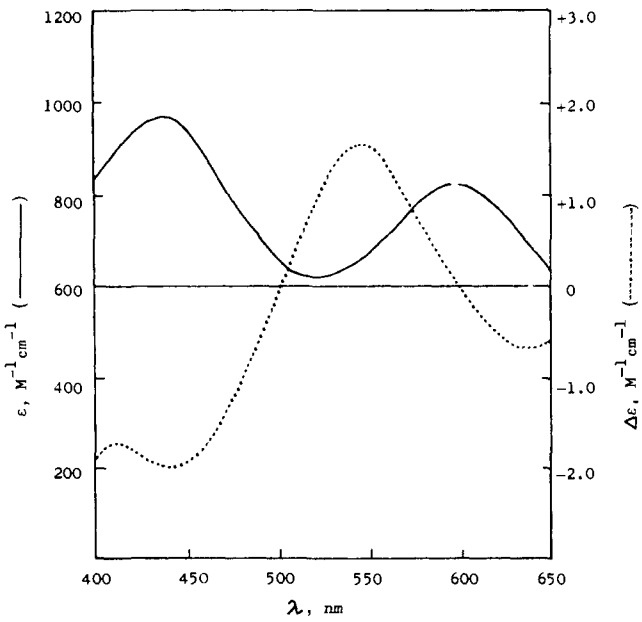


Figure 3. Visible and circular dichroism spectra of *N*-mercaptoacetyl-L-histidine–copper(II) complex. The spectra were obtained by mixing the ligand (1.0 mM) and $CuCl_2$ (1.0 mM) in borate buffer solution of pH 9.2. CD spectrum was recorded on a Jasco J-20 spectropolarimeter at 20 °C.

these visible bands, ranging from about 2.0×10^{-3} for the 440-nm band to about 0.8×10^{-3} for the 600-nm band. The 445- and 630-nm bands in the CD spectrum were used to determine $\Delta\epsilon/\epsilon$. The Kuhn's anisotropic factor, γ, can be expressed by the following equation: $\gamma = 4R/D$, where R and D are rotational strength and dipole strength, respectively. In a rough approximation, the following equation can be utilized to estimate the factor,

$$\gamma = |\Delta\epsilon/\epsilon|$$

where $\Delta\epsilon$ and ϵ are CD ($\epsilon_L - \epsilon_R$) and optical absorption in terms of extinction coefficients, respectively.[18] The ratio is typically $\geq 10^{-2}$ for magnetically allowed and electrically forbidden transitions of the ligand field (d–d) type. The 600-nm band is presumed, therefore, to be an electrically allowed charge transfer band. This presumption is consistent with the recent suggestion that there is a S → Cu(II) charge transfer for the intense 600-nm band of "blue" copper proteins.[2,3] In addition, the π charge transfer bands are expected to have a much larger value (>0.005) than is usually associated with the σ charge transfer bands. Accordingly, the 600-nm band of the MAH–Cu(II) complex may be assigned to the $\sigma(S) \rightarrow d(Cu)$ charge transfer transition. Solomon et al. have recently indicated that the 16000-cm^{-1} band in "blue" copper proteins exhibits a γ value well below 0.005, and such is attributed to a $\sigma S \rightarrow d_{x^2-y^2}$ charge transfer transition.[19] However, π and σ charge transfer can be distinguished by $\Delta\epsilon/\epsilon$

Table V. Visible Spectral Data for Sulfhydryl Containing Peptide-Co(II) Complexes and Co(II)-Substituted "Blue" Copper Proteins[a]

Ligand	Visible absorption maxima λ_{max}, nm (ϵ)
Co(II) plastocyanin[22]	510 (380), 630 (280). 675 (390)
Co(II) stellacyanin[22]	540 (320). 625 (380). 655 (460)
Co(II) azurin[22]	525 (220), 635 (340), 660 (290)
N-Mercaptoacetyl-L-histidine	560 (360), 603 (370). 668 (320)
3-Mercaptopropionyl-L-histidine	585 (300), 620 (430). 656 (410)
2-Mercaptopropionyl-L-cysteine	603 (450). 655 (500), 717 (470)
2-Mercaptopropionylglycine	610 (290). 675 (380), 730 (260)
3-Mercaptopropionylglycine	610 (330). 685 (450), 720 (430)
Glutathione	620 (240), 680 (370). 720 (280)
Glycylglycine	495 (15)
β-Alanyl-L-histidine	490 (20)

[a] The spectra were measured by mixing the ligand (4.0 mM) and $CoCl_2$ (2.0 mM) at pH 9.2 in a fully deaerated Tunberg tube.

values only in favorable cases, such as distorted tetrahedral D_{2d}. In a square-planar geometry it is possible that the 600-nm band is $\pi S \rightarrow d_{x^2-y^2}$, whereas the 440-nm peak could be $\sigma S \rightarrow d_{x^2-y^2}$. One would expect both π- and $\sigma S \rightarrow$ Cu(II) charge transfer to be at higher energies in square-planar complexes than in the blue copper proteins, if the latter possess nearly tetrahedral Cu(II) sites. The more intense band in the MAH-Cu(II) complex is at 438 nm, whereas the reverse is true in "blue" copper proteins. This fact suggests the possibility that the geometrical difference between the model complex and "blue" copper center may be responsible for the difference observed in energy. From resonance Raman studies, the trigonal-bipyramidal[2a] and flattened tetrahedral[2b] geometries have been proposed for "blue" copper sites. The CD spectrum of the MAH-Cu(II) complex shows three bands with the sign (− + −) above 440 nm. The CD spectra of laccase [Polyporus laccase: 469 ($\Delta\epsilon$ −0.9), 532 (1.2), and 615 (−3.0) nm;[20] Rhus vernicifera laccase: 446 (−2.4), 526 (3.0), and 633 (−5.6) nm[21]] resemble more closely that of the MAH-Cu(II) complex than those of the 2-MPC-Cu(II) complex [395 (−0.82) and 575 (0.59) nm] and glycyl-L-histidine-Cu(II) complex [485 (−0.16) and 565 (0.12) nm]. As to the copper hyperfine coupling constant in the parallel region, the sulfhydryl containing peptide-Cu(II) complexes such as MAH and 2-MPC have a close value to that of "blue" copper centers, where $A_{\parallel} < 100 \times 10^{-4}$ cm^{-1}. We have already indicated that the high covalency of the Cu-S bonding contributes to a decrease in the unpaired electron density on the Cu(II) atom.[4a] Furthermore, the 2:1 MAH-Co(II) complex showed absorption maxima at 560 (ϵ 360), 603 (370), and 668 (320) nm, and the spectral feature resembles appreciably those of Co(II) substituted "blue" copper proteins (see Table V). The MAH-Cu(II) complex, as a model for "blue" copper sites, serves to verify the speculation that the blue copper center involves cysteine sulfhydryl and histidine imidazole coordinations and that the intense 600-nm band is attributed to a $\sigma S \rightarrow$ d(Cu) charge transfer transition. All naturally occurring plastocyanins have a single cysteine residue and show a common amino acid sequence of Cys^{84}-X-Y-His^{87}.[16] The sequence includes Cys-Ser-Pro-His in the broad bean and elder plastocyanins, Cys-Ala-Pro-His in potato plastocyanin, and Cys-Glu-Pro-His in Chlorella plastocyanin. This can hardly be attributed to natural concidence. Thus, the MAH-Cu(II) complex, which has a $N_{Im}N_PS$ donor set (N_{Im} = imidazole nitrogen, N_P = peptide nitrogen, S = sulfhydryl sulfur), is of interest with regard to the quite recent suggestion for the coordination core ($N_{Im_2}N_PS$) of "blue" copper sites.[19]

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(7) The following abbreviations are used in this paper: DMPG = 2,3-dimercaptopropionylglycine; 2-MPC = 2-mercaptopropionyl-L-cysteine; MAH = *N*-mercaptoacetyl-L-histidine; 3-MPH = 3-mercaptopropionyl-L-histidine; 2-MPG = 2-mercaptopropionylglycine; 3-MPG = 3-mercaptopropionylglycine; CD = circular dichroism; ^{1}H NMR = proton nuclear magnetic resonance; ESR = electron spin resonance.
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